1965

# Inheritance of dwarf character in Medicago sativa L.

Thaddeus Hillery Busbice
*Iowa State University*

# INHERITANCE OF DWARF CHARACTER IN MEDICAGO SATIVA L.

by

Thaddeus Hillery Busbice

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Plant Breeding

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1965

TABLE OF CONTENTS

Page

INTRODUCTION ---------- 1

REVIEW OF LITERATURE ---------- 2

A Review of Genetic Studies ---------- 2

A Review of Cytological Studies ---------- 9

Variables in cytological behavior of autotetraploids --- 13

MATERIALS AND METHODS ---------- 19

Materials ---------- 19

Methods ---------- 23

EXPERIMENTAL RESULTS ---------- 25b

Part 1: The Inheritance of Dwarf Character in Source A ----- 25b

Test for tetrasomic inheritance ---------- 25b

Evidence for preferential pairing ---------- 29

Part 2: The Inheritance of Dwarf Character in Source B ----- 34

DISCUSSION ---------- 41

SUMMARY ---------- 43

LITERATURE CITED ---------- 44

ACKNOWLEDGMENTS ---------- 50

# INTRODUCTION

Alfalfa (Medicago sativa L.) is an economically important forage species and extensive efforts are being made by plant breeders to improve its value and productivity through hybridization and selection. An understanding of the genetic structure of an alfalfa population, the mechanics of meiosis, and the actions of genes is essential if continued improvement is to be made. The objective of this study was to obtain information about the mechanics of meiosis by observing the frequency of dwarf plants in segregating progenies from parents heterozygous for genes controlling dwarf phenotype.

Plant breeders are vitally interested in the rate at which genes segregate. This rate is dependent upon the events that take place during meiosis and gamete formation. The probability of obtaining useful segregates from a hybridization program is dependent upon these events and the degree of precision with which they occur. The genetic variation for quantitative characters, such as yield, found within a population also is dependent to large extent upon the events of meiosis.

The genetic expectations of the classical geneticist as well as the statistical methods of quantitative geneticist and plant breeder are all based upon a hypothesis about the mechanics of meiosis. The accuracy and usefulness of these expectations and methods depend upon the accuracy of the hypothesis. In this study the hypothesis was made that alfalfa behaves as an autotetraploid and the four homologs pair at random. The data were tested for agreement with this hypothesis.

# REVIEW OF THE LITERATURE

The somatic cells of Medicago sativa L. each contain 32 chromosomes. The preponderance of genetic and cytological data indicates autotetraploid behavior. Many papers have been published dealing with the theoretical and practical significance of autoploidy in genetic and breeding problems: Haldane (1930), Bartlett and Haldane (1934), Mather (1935, 1936), Little (1945, 1958), Comstock (1952), Kempthorne (1955), Catcheside (1956), Li (1957), Carnahan (1960), Burnham (1962), Demarly (1963), Levings and Dudley (1963), Dudley (1964), Buzzell (1965), Busbice and Wilsie (1966).

## A Review of Genetic Studies

Table 1 is a summary of genetic studies that have been conducted on alfalfa. Until 1951 only one group of investigators had reported tetrasomic inheritance. Tysdal, Kiesselback and Westover (1942) demonstrated that Korohoda's (1933) data on leaf shape would fit the expected ratios under tetrasomic inheritance. They pointed out that, in many cases, the genetic ratios that had been reported would fit the expected segregation in an autotetraploid better than the complicated disomic ratios that had been suggested. After surveying the genetic and cytological evidence that had accumulated up to that time, they concluded that it was highly probable that common alfalfa is a tetraploid and since many of the data were conflicting suggested that it may be an intermediate between a true alloploid and true autoploid.

In 1951 Stanford (1951) reported a clear-cut case of tetrasomic inheritance. He stressed the importance of studying the $F_3$ segregating

Table 1. Summary of genetic studies in Medicago sativa L.[a]

| Author and reference | Character | Authors' interpretation | d or t[b] |
|---|---|---|---|
| Hagem (1919) | Flower color | 2 factors, A and B for violet. Color non-fading only when both homozygous dominant; otherwise it fades. One factor, C for yellow color | d |
| Waldron (1925) | Albino (flower) no purple veining, foliage normal | More than three factors | d |
| | White seed coat | Same as albino flower (see above) | d |
| Waldron (1929) | Flower color | 3 factors for purple | d |
| Korohoda (1933) | Flower color | 4 factors, one each for cream, blue, and violet, and one (or two) intensifying these colors | d |
| | Stem color green vs. colored | Recessive factor for color | d |
| | Stem structure falcata type vs. sativa type | Duplicate factors | d |
| Peltier and Tysdal (1934) | Wilt resistance | Possibly three factors for resistance | d |
| MacVicar (1935) | White seed coat | Single recessive factor for white seed | d |
| | Black seed coat | 2 factors, 2 modifiers | d |
| Burton (1937a and b) | Flower color | 3 factors for purple | d |

[a] A portion of this summary was adapted from Atwood, S. S. and Grun, P. (1951, p. 158).

[b] d = disomic, t = tetrasomic.

Table 1. (Continued)

| Author and reference | Character | Author's interpretation | d or t[b] |
|---|---|---|---|
| Burkart (1937) | Flower color | 3 factors for purple | d |
| Bauder (1938) | Leaf abnormality, normal vs. odd | 2 dominant factors, 0 and I with I being an inhibitor epistatic to 0 | d |
| Odland and Lepper (1939) | Leaf abnormality Normal vs. crinkle | 2 complementary dominant factor for normal epistatic to dominant Cr. | d |
| Lepper and Odland (1939) | Flower color Purple vs. white | 2 factors for purple | d |
| | Purple vs. yellow | 3 factors | d |
| | Yellow vs.white | 3 factors | d |
| | Variegated vs. white | 4 factors for color | d |
| Armstrong and Gibson (1941) | Flower color | 3 factors for purple, one factor for cream allelic to one of the factors for purple | d |
| | Pod hairiness | One dominant factor for upright, plus an inhibitor | d |
| | Stoloniferous | More than 2 factors with "stoloniferous" tendency recessive | d |
| Tysdal, Kiesselback and Westover (1942) | Using Korohoda's (1933) data for leaf shape | Single gene, falcata type dominant, sativa type recessive | t |
| Schröck (1943) | Flower color | 1 factor for yellow<br>1 factor for pigment<br>1 factor for pattern | d<br>d<br>d |
| | Calyx color pigment vs. no pigment | 1 factor | d |
| | Flower size large vs. small | 1 factor | d |

Table 1. (Continued)

| Author and reference | Character | Author's interpretation | d or t[b] |
|---|---|---|---|
| Schröck (1943) (Continued) | Leaf color dark vs. light | 1 factor | d |
| | Leaf abnormality folded vs. flat | 1 factor | d |
| | Abnormal growth normal vs. stunted | 1 factor | d |
| Wilson (1947) | Wilt resistance | 3 or possibly 4, partially dominant genes | d |
| Govas and Fernandez (1947) | Anthocyanin in seedlings and flowers | Same genes for seedling anthocyanin and flower color | d |
| | Compressed racemes | Single recessive gene | d |
| Weihing (1948) | Flower color | 3 factors for purple vs. white<br>3 factors for yellow vs. white<br>plus complementary factor | d |
| Stanford (1951) | Flower color | 1 dominant gene for purple color | t |
| Stanford and Cleveland (1954) | Folded leaf abnormality | 1 recessive gene | t |
| | Mottled leaf abnormality | 1 recessive gene | t |
| Fyfe and Wills (1955) | Albino | 1 recessive gene | t |
| Twamley (1955) | Flower color | One of 2 factors for purple appeared to follow tetrasomic pattern exclusively, whereas the second factor followed a disomic pattern in some plants and a tetrasomic in others | t and d |

Table 1. (Continued)

| Author and reference | Character | Author's interpretation | d or t[b] |
|---|---|---|---|
| Davis (1956) | Elongated hypocotyl | 1 recessive gene | t |
| Dudley and Wilsie (1956) | Branched inflorescence abnormality | 2 recessive genes, one tetrasomic and the other disomic | t and d |
| | Vestigal flower abnormality | 2 recessive genes, one tetrasomic and the other disomic | t and d |
| Lewis and Elling (1956) | Lethal character | 2 complementary dominant genes | t |
| Oldemeyer (1956) | White seed | 2 factors, one recessive and the other dominant, tetrasomic inheritance with evidence of preferential pairing | t |
| Lesins (1957) | Purple flower color | 1 dominant gene | t |
| Markus and Wilsie (1957) | Exposed stigma abnormality | 1 recessive gene | t |
| Stargaard (1957) | Flower color | Tetrasomic inheritance or tetrasomic-disomic inheritance fit the data | t or t-d |
| Cleveland and Stanford (1959) | Leaf abnormality | 1 recessive gene | t |
| Stanford (1959) | Zebra leaf | A single tetrasomic gene with dominance at the duplex level | t |
| Childers and McLennan (1960) | Complete male sterile | 3 recessive factors | d |
| Goplen and Stanford (1960) | Resistance to Meloidogyne hapla | 1 dominant gene | t |
| Childers and McLennan (1961) | Chlorophyll-deficient | 1 recessive gene | t |

Table 1. (Continued)

| Author and reference | Character | Author's interpretation | d or t[b] |
|---|---|---|---|
| Dessureaux (1961) | Yellow cotyledon | Recessive genes | t |
| Childers (1962) | Yellow leaf abnormality | 1 recessive gene | t |
| Murray and Craig (1962) | Cauliflower-head and single leaf mutant | 1 recessive gene controls both characters | t |
| Soudah (1962) | Purple flower color | 1 gene with dosage effect, AAAA=very deep purple AAAa=deep purple AAaa=purple Aaaa=light purple aaaa=pure white | t |
| Whittington and Burrage (1963) | Ruptured epidermis | Complicated by variable penetrance - appears to be due to a single tetrasomic gene expressed when two or more mutant alleles are present | t |
| Barnes and Hovin (1965) | Pale-green plant | 1 recessive gene | t |
| Pedersen and Barnes (1965) | Downy mildew resistance | 1 gene with incomplete dominance | t |
| Stanford (1965) | Sticky leaf | 1 recessive gene | t |

generation before drawing definite conclusions as to whether the data indicates tetrasomic or disomic inheritance. A duplex plant (AAaa) segregating approximately 35:1 in accordance with tetraploid theory could be mistaken for a 15:1 or 63:1 ratio in accordance with diploid theory by considering 2 or 3 factors (AaBb or AaBbCc). Likewise a simplex plant (Aaaa) will segregate approximately 3:1 just as a diploid plant (Aa). Since Stanford's paper only one case of exclusively disomic inheritance has been reported. Childers and McLennan (1960) found evidence for disomic inheritance for a complete male sterile factor, however, the data were not entirely conclusive. Twamley (1955), Dudley and Wilsie (1956), and Stargaard (1957) have reported evidence of both tetrasomic and disomic inheritance affecting a character. Each character studied involved more than one gene.

Stanford (1951) pointed out that many of the early studies were not complete enough to distinguish between disomic and tetrasomic inheritance.

Oldmeyer (1956) suggested that long established alfalfa varieties may display disomic inheritance while varieties of more recent origin may display tetrasomic inheritance. He crossed a white-seeded Medicago sativa plant to a normal tan-seed M. sativa plant and also to a colchicine induced autotetraploid M. falcata plant. Ratios characteristic of preferential pairing (a tendency toward disomic inheritance) were obtained from pure M. sativa hybrids and ratios characteristic of random chromosome pairing were obtained from the white-seed M. sativa X autotetraploid M. falcata hybrids. He hypothesized that improvement of the long established alfalfa varieties has been primarily by intravarietal

selection and, therefore, any chromosome differentiation existing in these varieties has not been changed. In recent years, however, alfalfa varieties have been synthesized from strains more diverse in origin (incorporating M. falcata germ plasm). The sub-genomes from these unrelated strains would lose their identity in a synthetic variety, resulting in random pairing of the four homologous chromosomes accounting for the recent reports of inheritance based upon random chromosome pairing.

A recent report on the inheritance of a quantitative character indicates that tetrasomic inheritance is the rule in alfalfa. Busbice and Wilsie (1966) compared the forage yields of full sib progenies ($S_1$ X $S_1$) and backcross progenies ($S_1$ X $S_0$) and found highly significant differences. They concluded that quantitative characters such as yield also are inherited tetrasomically since differences would be expected under tetrasomic inheritance and no differences would be expected under disomic inheritance.

## A Review of Cytological Studies

Ledingham (1940) found that homologous chromosomes of Medicago sativa and M. falcata interpair freely, suggesting no cytological criterion by which the forms can be separated into two distinct species.

Julen (1944) observed that "triploids" (actually hexaploids in that they contain 8 X 6 = 48 chromosomes) had a remarkably regular meiosis judging from the low univalent frequency (1-2 univalents in 29% of the cells) and the absence of multivalents. Up to 24 bivalents could be formed within the "triploids". The meiosis in the "triploids" afforded

good support for the view that M. sativa is already a tetraploid in its spontaneous form. Meiosis in the "diploid" corroborated this assumption; besides bivalents there occurred univalents, trivalents as well as quadrivalents.

Grun (1951) reported an average of 40 percent of the cells studied in M. sativa contained one or more quadrivalents, and 1 percent contained as many as four, indicating a possible degree of autoploidy. From limited data Hanson (1952) could distinguish less than 10 percent of the cells by the presence of one or more quadrivalents.

Lesins (1952) described a haploid plant (16 chromosomes in the somatic cell) of the Grimm variety and its crosses to M. falcata. The haploid was weaker than normal plants and some morphological abnormalities were noted. Two hybrids were obtained in crosses with M. falcata (2n = 16), one of which was fully fertile when backcrossed to M. falcata plants. It was concluded that the progenitor of the haploid plant contained four closely related genomes that had become weakened by the accumulation of lethal genic and cryptic structural changes under the tetraploid condition.

Oldemeyer and Brink (1953) studied the fertility of hybrids between autotetraploids derived from diploid M. falcata and plants of the Cossack variety (a tetraploid variety that contains both M. sativa and M. falcata germ plasm). The hybrids formed seed as freely as the Cossack control while the autotetraploid M. falcata was distincly less fertile. These results demonstrated that the haploid complement (n = 8) occurring in diploid M. falcata, when duplicated, may be substituted for one of

the two homologous sets of chromosomes present in Cossack without impairing seed fertility. The results were interpreted to support the view that the cultivated alfalfas are essentially autotetraploids.

Armstrong (1954) reported evidence that the genomes of M. sativa are only partially homologous. Cytological studies were made of an induced octoploid and of a hexaploid, obtained from crosses of tetraploid by octoploid. Fairly complete pairing in the hexaploid supported the view of a high degree of homology. However, the quadrivalent frequency in the octoploid was more than three times as high as in the tetraploid which suggests some lack of homology among the genomes. He advanced the theory that tetraploid Medicago originated from crosses between a series of diploid species fairly similar cytologically but differing in well marked, morphological characters.

Stanford and Clement (1958) observed meiosis in a haploid alfalfa plant. Good pairing at metaphase indicated that common alfalfa is essentially an autotetraploid in which only minor differences in chromosome sets have developed. They concluded that tetrasomic ratios should be the rule, but the possibility of disomic ratios associated with certain chromosomes is not excluded.

Cleveland and Stanford (1959) observed chromosome pairing in M. sativa, in induced autotetraploid M. falcata and in their $F_1$ hybrids. They found multivalent frequencies were lower by approximately one multivalent per cell in M. sativa than in tetraploid $F_1$ hybrids and autoploid M. falcata. The four sets of chromosomes in M. sativa had less tendency to pair as quadrivalents in prophase, or a greater tendency to dissociate from

quadrivalent into bivalents before metaphase I. This suggested that a deviation from true autotetraploid behavior exists in meiosis of M. sativa. They hypothesized that the M. sativa chromosomes when present in the M. sativa complement, where structural differences would be homozygous, would have a tendency to pair selectively as bivalents.

Another hypothesis considered was that a balanced genetic constitution controling the processes which inhibit multivalent formation is present in M. sativa. Assuming that M. sativa arose as an autotetraploid, such a genetic constitution could have been built up in response to natural selection for higher fertility. The higher multivalent frequencies in the $F_1$ hybrid could then be explained as the result of an undefined breakdown of the genic balance. It is also possible that alfalfa originated as a hybrid between species of Medicago which have some structural differences in the chromosome complements, but a high degree of structural similarity. Cleveland and Stanford concluded that whatever the origin of alfalfa, the species as it exists today, deviates in chromosome behavior from that of a true autoploid.

Clement and Stanford (1961) described the chromosomes of alfalfa at pachytene. The chromosomes are characterized by prominent knobs on one end of each chromosome. The knobbed arms are highly chromatic, whereas the arms without knobs are largely achromatic except for the regions adjacent to the centromeres. The knobbed arms are shorter in chromosomes with submedian centromeres. In diploid alfalfa, rod shaped bivalents are usually formed at metaphase and ring bivalents occur infrequently. Cultivated tetraploid alfalfa as well as induced autotetraploid alfalfa form few multivalents at metaphase (1-3 average). Observations indicated

that chiasmata frequencies are much reduced in one arm (presumably the shorter chromatic arm). This may explain the low frequency of multivalents in the tetraploid form and a predominance of rod bivalents in the diploid form of alfalfa.

Clement and Lehman (1962) found very close pairing at pachytene and metaphase in a dihaploid (somatic chromosome number equal 16). This apparent close homology was taken as further indication of the near autotetraploid nature of alfalfa.

Gilles and Randolph (1951) studied the relative frequency of quadrivalent and bivalent association of the chromosomes in a strain of autotetraploid maize (Zea mays) at the beginning and end of a 10 year period. There were fewer quadrivalents and more bivalents at the diakinesis stage of meiosis at the end of the 10 year period than there were at the beginning of the period, the average frequencies being 7.46 and 8.47, respectively. The results suggested that autoploids, which form multivalents with relatively high frequency at the time of their origin, may shift to the bivalent type of synapsis that is characteristic of most alloploids. They concluded that the presence or absence of quadrivalent association of the chromosomes in natural polyploids may not be a reliable criterion for determining their manner of origin by autoploidy or alloploidy.

## Variables in cytological behavior of autotetraploids

Little (1945, 1958) lists three main variables in cytological behavior which may affect the proportions of recessives found in a segregating progeny (mode of pairing, quadrivalent formation and chiasma frequency) and gives the following discussion. Pairing in a tetraploid may be

classified as either random or selective with reference to any given group of four homologous chromosomes. Even with the same tetraploid, some members of a genome may undergo random pairing, while others may display varying degrees of selective pairing.

Selective pairing occurs when the four homologs are not equally homologous but tend to fall into groups such that the two chromosomes within a group display more affinity than two chromosomes from different groups. Homology is a relative term and can vary from absolute identity, such as found in doubled haploids, to the very weak homology found in secondary pairing. As a result, pairing in tetraploids can vary from completely random, where the four chromosomes are equally homologous, to completely selective, where plants behave functionally as a diploid. All degrees of selective pairing between these two extremes may exist. With complete selective pairing a duplex (AAaa) may be non-segregating if the pairing is $A_1A_1$ and $a_2a_2$ or it may segregate 16:1 if the pairing is $A_1a_1$ and $A_2a_2$.

Buzzell (1965) has produced a very good theoretical discussion on preferential pairing. Table 2 is taken from his paper. Buzzell defines the following terms:

Homogenic pairing: Similar alleles pair

Homogenic dyads: Contain similar alleles and produce homogenic gametes

Homogenic gametes: Contain similar alleles

Heterogenic pairing: Dissimilar alleles pair

Heterogenic dyads: Contain dissimilar alleles and produce homogenic and heterogenic gametes in equal frequencies

Heterogenic gametes: Contain dissimilar alleles.

Table 2. Expected genetic ratios for a $B_1B_1b_2b_2$ or BBbb or $B_1b_1B_2b_2$ genotype with varying degrees of homogenic and heterogenic pairing and with dominance at the single-dosage level[a]

| $H_o$:$H_e$ | Selfed or crossed to $B_1B_1b_2b_2$ or BBbb or $B_1b_1B_2b_2$ | | | | | | | | | | | | Crossed to: | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1:0 | .9:.1 | .8:.2 | .7:.3 | .6:.4 | .5:.5 | .4:.6 | 1/3:2/3 | .3:.7 | .2:.8 | .1:.9 | 0:1 | Bbbb | bbbb |
| 1:0 | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| .9:.1 | 1599.00** | 799.00 | 532.33 | 399.00 | 319.00 | 265.67 | 239.00 | 227.57 | 199.00 | 176.78 | 159.00 | 79.00 | 39.00 | |
| .8:.2 | | 399.00 | 265.67 | 199.00 | 159.00 | 132.33 | 119.00 | 113.29 | 99.00 | 87.89 | 79.00 | 39.00 | 19.00 | |
| .7:.3 | | | 176.78 | 132.33 | 105.67 | 87.89 | 79.00 | 75.19 | 65.67 | 58.26 | 52.33 | 25.67 | 12.33 | |
| .6:.4 | | | | 99.00 | 79.00 | 65.67 | 59.00 | 56.14 | 49.00 | 43.44 | 39.00 | 19.00 | 9.00 | |
| .5:.5 | | | | | 63.00 | 52.33 | 47.00 | 44.71 | 39.00 | 34.56 | 31.00 | 15.00 | 7.00 | |
| .4:.6 | | | | | | 43.44 | 39.00 | 37.10 | 32.33 | 28.63 | 25.67 | 12.33 | 5.67 | |
| 1/3:2/3 | | | | | | | 35.00 | 33.29 | 29.00 | 25.67 | 23.00 | 11.00 | 5.00 | |
| .3:.7 | | | | | | | | 31.65 | 27.57 | 24.40 | 21.86 | 10.43 | 4.71 | |
| .2:.8 | | | | | | | | | 24.00 | 21.22 | 19.00 | 9.00 | 4.00 | |
| .1:.9 | | | | | | | | | | 18.75 | 16.78 | 7.89 | 3.44 | |
| 0:1 | | | | | | | | | | | 15.00 | 7.00 | 3.00 | |

[a]Source: Buzzell (1965)

*Infinity:0

**1599.00:1, 799.00:1, etc.

Preferential pairing, which may be either homogenic or heterogenic, is assumed to result from genic and/or chromosomal affinities. In Table 2, $H_o$:$H_e$ is the ratio of homogenic pairing to heterogenic pairing. One may observe that with varying degrees of preferential pairing almost any ratio may be expected. When random pairing among the homologs occurs, the ratio of $H_o$:$H_e$ is 1/3:2/3.

Doyle (1963) observed preferential pairing in structural heterozygotes of Zea mays. The known structural rearrangement used in this study was In3a, a paracentic inversion in the long arm of chromosome 3 of maize. The inversion occupied about one-third of the total chromosome length. Gene segregation data of normal controls and of inversion heterozygotes were found to be significantly different and in conformity with theoretical expectations. In trisomics (Aaa) the frequency of heterogenetic bivalents was estimated at 26.2 percent instead of the random 66.7 percent value.

In the tetraploid it was possible to estimate the value of heterosynapsis to be 22.8 percent by the use of data on the anaphase bridge frequency of simplex (InNNN) and duplex (InInNN) tetraploids.

Shaver (1963) by mating appropriate stocks of 4n maize with 4n perennial teosinte, produced two types of allotetraploids, one carrying inversion 3a as a structurally heterozygous region, and the other without structural hybridity. Similarly, structurally heterozygous and non-heterozygous autotetraploids of pure maize were produced. The genetic effect of the defined chromosome rearrangement on preferential segregation was measured from test cross ratios of two linked gene markers in the loop. In the allotetraploid, the average segregation ratio for the

contained markers was altered from 10.6:1 to 31.2:1 by the insertion of structural hybridity. In the autotetraploid the average test cross ratio was altered from 5.7:1 to 8.0:1. There were significant differences among the progenies of different plants of the same genotype.

Menzel (1964) found that in an allotetraploid of the intergeneric hybrid, Lycopersicon esculentum X Solanum lycopersicoides, the chromosomes exhibited a very high degree of preferential pairing at pachytene, despite the fact that homoeologues synapsed almost perfectly at pachytene in the corresponding $F_1$ hybrid. Preferential pairing was shown to be due to highly non-random synapsis rather than to preferential chiasma formation. The ability to discriminate exact homologues from homoeologues seemed to be uniformly distributed along chromosomes and not attributed to differential heterochromatinization or to a linear arrangement long enough to be visible at pachytene.

Twamley (1955), when studying flower color inheritance in alfalfa, observed some families showing wide ratios (of the order of 100 purple: 1 white) upon selfing and could not determine whether the small number of non-purple segregates was due to disomic segregation of triplicate factors or to a semi-random type of pairing.

Buzzell and Wilsie (1963) suggested preferential pairing of a homogenic nature to account for an excess of brown keel tips in duplex plants in Lotus corniculatus.

As noted earlier, Little (1945) listed quadrivalent formation and chiasma frequency as variables in the cytological behavior of autotetraploids. These two events must occur if there is double reduction, a

phenomenon first described mathematically by Mather (1935). An index of separation (a quantitative measure of double reduction) proposed by Mather was shown to be dependent upon the parameters a and e. If quadrivalents are never formed, a = 0. If quadrivalents are always formed, a can reach its maximum of 1/3. If there is no crossing over between the locus and the centromere e = 0. If there is at least 1 crossover between the locus and the centromere, e can reach its maximum value of 1. The maximum value for the index of separation is ae = 1/3, the minimum value ae = 0. The results of double reduction is an excess of recessive genotypes in a segregating progeny. For an understanding of the biological basis of double reduction the reader is referred to Burnham (1962).

In addition to preferential pairing and double reduction, numerical non-disjunction can also alter the frequency of recessives in a segregating progeny. Catcheside (1956) stated that there can be no doubt that non-disjunction makes an appreciable contribution to gametic formation and that the effects of it have previously been confused with the effects of double reduction. He presented a mathematical treatment of this phenomenon. Burnham (1962) has summarized cytological data on maize which confirms a high frequency of non-disjunction manifest in a high frequency of aneuploid plants.

# MATERIALS AND METHODS

## Materials

Two sources of plant material, designated A and B, from Medicago sativa L. were studied. Segregating progenies from both of these sources contained plants with dwarf phenotypes. The dwarfs were characterized by shortened internodes and darker green foliage.

Source A was Clone 936-16, an $F_1$ from a 'DuPuits' X 'Vernal' cross. The DuPuits parent was Clone 840 which originated from sib-mating $S_1$ selections of the DuPuits variety. The Vernal parent was Clone 631-67-52, a selection from an $S_1$ progeny of 'Vernal X-rayed'. Clone 936-16 had a completely normal phenotype, but segregated, upon selfing, approximately 40 normal:1 dwarf.

Source B was seven $F_1$ plants from a cross of Dwarf X Clone 538-8-1. The dwarf parent was observed as a mutant in the DuPuits variety and Clone 538-8-1 was an $S_2$ selection from DuPuits. The phenotypes of these $F_1$ plants were completely normal.

When crossed with unrelated normal plants, the dwarfs from both sources produced only normal $F_1$ progenies indicating that the dwarf phenotype is conditioned by a recessive gene or genes in the homozygous condition.

When dwarfs from source A were crossed with dwarfs from source B only normal offspring were produced. $F_2$ progenies, obtained by selfing these $F_1$'s, segregated in ratios that ranged from 0.80 normal:1 dwarf to all normal. This indicated that two genetic systems were involved and

for this reason the genetics of sources A and B sere studied as two separate problems. No further attempt was made to determine the genetic relationship of the two systems.

Both the normal and dwarf plants from source A were completely male and female fertile. The flowers of the dwarf plants were normal and produced an abundance of viable pollen. Self-fertility of Clone 936-16 was low (40 seeds per 100 flowers tripped) and with inbreeding the self-fertility of its progeny was much lower.

In contrast, the seven $F_1$ plants of source B were completely male and female fertile and highly self-fertile (100 to 200 seed per 100 flowers tripped). However, this high self-fertility was greatly reduced by the inbreeding incident to the genetic study. All of the dwarf segregates from source B were female fertile but completely male sterile in that they produced no viable pollen.

When treated with gibberellic acid, dwarfs from both sources A and B produced normal phenotypes. The foliage of the dwarf plants was wet at weekly intervals with an aqueous solution, containing 100 PPM gibberellic acid. After 4 weeks some of the dwarfs could not be distinguished from the normal controls.

Figures 1 and 2 show the dwarf phenotype in the seedling and flowering stage and also after treatment with gibberellic acid. There was no difficulty in distinguishing dwarf and normal plants in the seedling stage. The petiole of the first leaf of the dwarf was very short in comparison to the normal seedlings and the cotyledons of the dwarf were darker green than the normal.

Figure 1. (A) dwarf plant in flowering stage from source B. (B) dwarf genotype from source B that has been treated with gibberellic acid. (C) normal plant from source B. (D) dwarf plant in flowering from source A. (E) dwarf genotype from source A that has been treated with gibberellic acid. (F) normal plant from source A. (G) segregating progenies growing in greenhouse flat, toothpicks indicate dwarf.

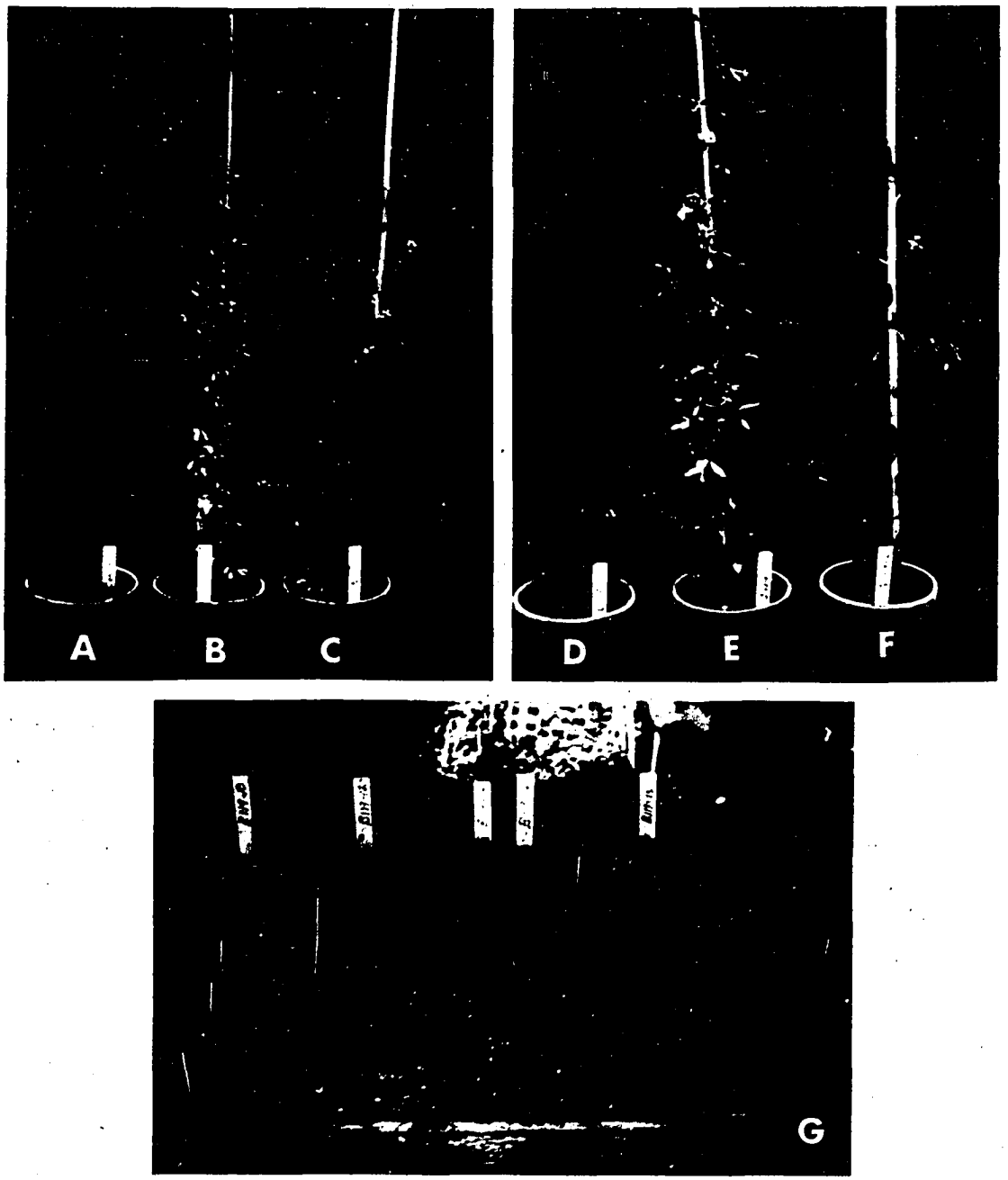
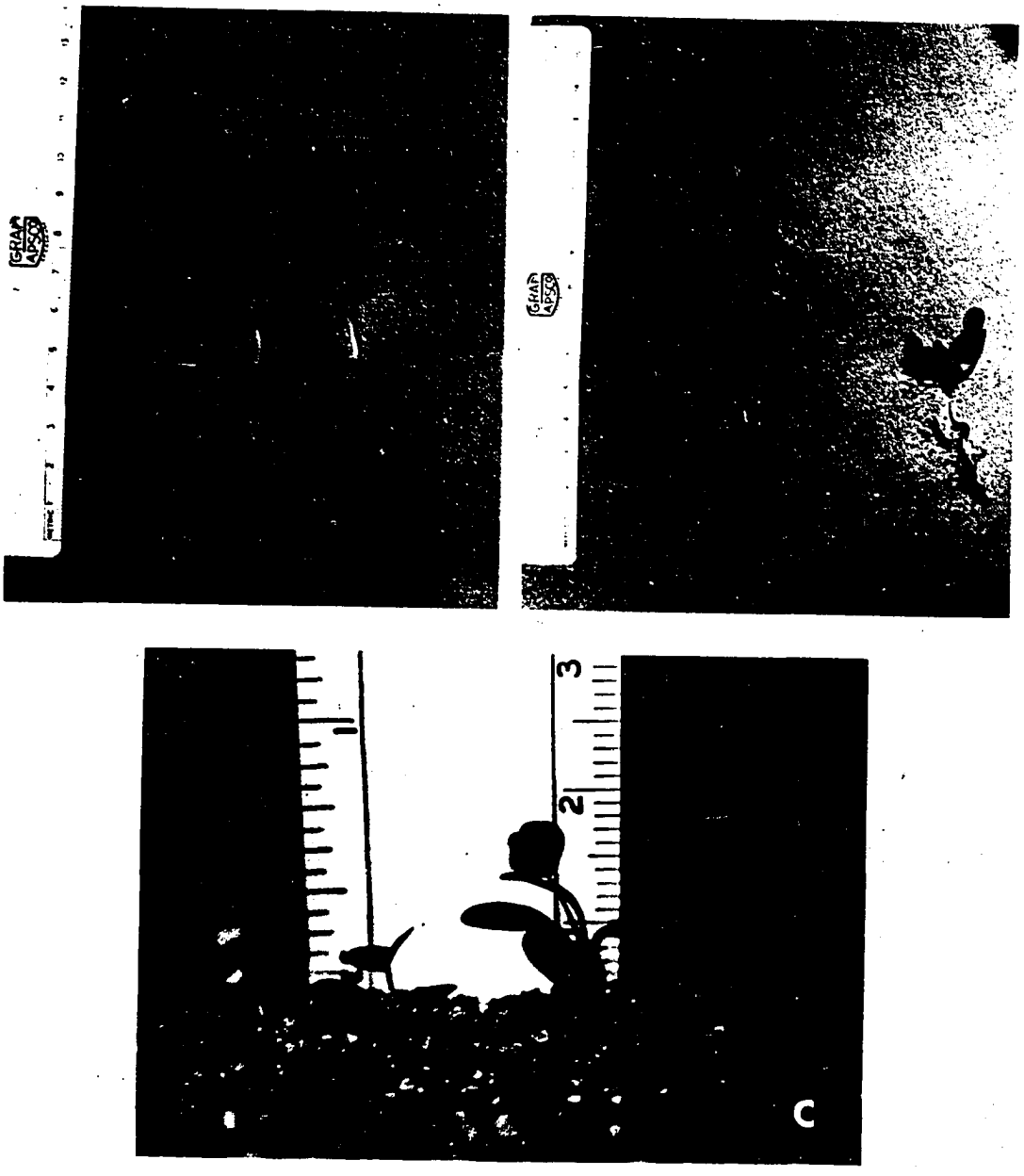

A
B
C
D
E
F
G

Figure 2. (A) normal seedling on left, dwarf seedling on right 10 days after emergence. (B) normal seedlings on left and dwarf seedling on right approximately 3½ weeks after germination. (C) dwarf seedling on left and normal seeding on right 10 days after emergence. All the seedlings shown are from source B, seedings from source A are very similar.


GRAF APSCO
GRAF APSCO
1
2
3
C

## Methods

The entire study was conducted in the greenhouse.

In all crosses, the seed parents were emasculated by sucking the pollen from around the stigma with the aid of a vacuum pump. An excess of pollen from the male parent was placed upon the stigma of the seed parent immediately after emasculation.

Self seed were produced by tripping the flowers with the end of a flat toothpick. This allowed self pollination. Care was taken to prevent unwanted crossing with other plants.

All the seed produced from selfing and crossing were germinated in greenhouse flats filled with sterilized soil. The seedlings were rated as normal or dwarf within 10 days after germination. There was no difficulty in identifying the dwarfs at this stage of growth.

It was recognized that inheritance studies that involve several genes affecting one character may be very complex and difficult to interpret, especially if tetrasomic inheritance is involved. The purpose of this study was not to make a complete analysis of dwarf inheritance but to obtain information pertaining to the mechanics of meiosis. This can best be accomplished by observing the segregation of one gene affecting a character. For this reason, plants were sought that segregated 3 normal:1 dwarf upon selfing. Such a plant is clearly heterozygous for but one recessive gene controlling dwarf phenotype. If there is disomic inheritance the zygotic array of the selfed progeny of such an individual is ($\frac{1}{4}$AA + $\frac{1}{2}$Aa + $\frac{1}{4}$aa). If there is tetrasomic inheritance the zygotic array of the selfed progeny of such an individual is ($\frac{1}{4}$AAaa + $\frac{1}{2}$Aaaa +

¼aaaa). By selfing or test crossing the selfed progeny of an individual that segregates 3 normal:1 dwarf, one can draw definite conclusions as to whether alfalfa behaves as a diploid or tetraploid. Also, from data such as these, it may be possible to detect preferential pairing, double reduction, or numerical non-disjunction.

The published literature suggested that tetrasomic inheritance is to be expected in alfalfa. Therefore, the null hypothesis in this study was that alfalfa behaves as an autotetraploid and displays random chromosome segregation. The data were tested for agreement with this hypothesis and a probability of .05 was established as the level at which the hypothesis would be rejected. An attempt was made to collect sufficient data, not only to distinguish between disomic and tetrasomic inheritance, but also to detect slight deviations from random chromosome segregation.

With tetrasomic segregation it is necessary to distinguish between simplex plants (Aaaa) and duplex plants (AAaa) by their self and backcross ratios. One must grow progenies of adequate size to distinguish between a 3:1 ratio and a 35:1 ratio, for self progenies, and between a 1:1 and 5:1 ratio for backcross progenies. According to Mather (1957) a minimum of 18 plants must be grown to make these distinctions at the .05 level of probability. The ambiguous ratio for 3:1 and 35:1 ratios, that is, a ratio that will fit either hypothesis with equal probability, is 10.247 normal:1 dwarf. The ambiguous ratio for 1:1 and 5:1 ratios is 2.236 normal:1 dwarf. In this study at least 18 plants were grown in every segregating progeny. There was no difficulty in distinguishing between the various ratios.

This investigation was divided into two parts: (1) the inheritance of dwarf character in source A, and (2) the inheritance of dwarf character in source B. Although the phenotype is similar in both sources, dwarf A and dwarf B can be considered as two separate characters.

# EXPERIMENTAL RESULTS

## Part 1: The Inheritance of Dwarf Character in Source A

### Test for tetrasomic inheritance

Clone 936-16 segregated, upon selfing, 40.64 normal:1 dwarf indicating that the dwarf character is conditioned by one or more recessive genes. In Table 3 it can be seen that the observed ratio fits the expected ratio of a duplex (AAaa) plant under the hypothesis of random chromosome segregation.

Several $F_1$ plants from a cross (dwarf ♀ X Normal ♂) also were selfed. The normal parent was Clone 1317, a selection from the Vernal variety, and the dwarf was a segregate of Clone 936-16. These ratios also are given in Table 3. These data, combined with the data from Clone 936-16, support the hypothesis of tetrasomic inheritance.

Backcross data from Clone 936-16 X Dwarf are presented in Table 4. The data fit the 5 normal:1 dwarf ratio expected under random chromosome segregation considering Clone 936-16 to be duplex (AAaa) and the dwarf to be nulliplex (aaaa).

A plant that segregates upon selfing 3 normal:1 dwarf was needed for a positive test for tetrasomic inheritance. With disomic inheritance, the self progeny of such a plant would be expected to be composed of ¼ normal non-segregating plants (AA) + ½ normal plants (Aa) that would segregate 1 normal:1 dwarf, when backcrossed to a dwarf, + ¼ non-segregating dwarf plants (aa). With tetrasomic inheritance, the self progeny would be expected to be composed of ¼ normal plants (AAaa), that would segregate 5 normal:1 dwarf when backcrossed to a dwarf, + ½ normal plants (Aaaa), that would segregate

Table 3. Genetic analysis of self progenies from normal plants that were heterozygous for a recessive gene controlling dwarf phenotype

| | Progenies segregating approximately 35 normal:1 dwarf | | | | |
|---|---|---|---|---|---|
| | Normal | Dwarf | Observed ratio | $X^2$ | P |
| Clone 936 - 16 | 894 | 22 | 40.64:1 | .534 | .50-.30 |
| $F_1$'s of (Dwarf X Normal) | | | | | |
| A200 | 77 | 2 | 38.50:1 | .021 | .90-.80 |
| A202 | 193 | 9 | 21.44:1 | 2.028 | .20-.10 |
| A203 | 398 | 13 | 30.62:1 | .199 | .70-.50 |
| A204 | 117 | 4 | 29.25:1 | .112 | .80-.70 |
| A205 | 309 | 11 | 28.09:1 | .477 | .50-.30 |
| Summary | 1988 | 61 | 32.59:1 | .237 | .70-.50 |
| Test for homogeneity: 5 degrees of freedom | | 3.371 - .237 = | | 3.134 | .70-.50 |
| | Progeny segregating approximately 3 normal:1 dwarf | | | | |
| A136 | 137 | 45 | 3.04:1 | .007 | .95-.90 |

Table 4. Genetic analysis of backcross progenies

| | Normal | Dwarf | Observed ratio | $X^2$ | P |
|---|---|---|---|---|---|
| **Progenies segregating approximately 5 normal:1 dwarf** | | | | | |
| Dwarf ♀ X 936-16♂ | 239 | 63 | 3.79:1 | 3.761 | .10-.05 |
| 936-16 ♀ X dwarf♂ | 115 | 25 | 4.60:1 | .114 | .80-.70 |
| Summary | 354 | 88 | 4.02:1 | 3.275 | .10-.05 |
| Test for homogeneity: 1 degree of freedom | 3.875 - 3.275 = | | | .600 | .50-.30 |
| **Progenies segregating approximately 1 normal:1 dwarf** | | | | | |
| A136 ♀ X dwarf♂ | 23 | 13 | 1.77:1 | 2.777 | .10-.05 |
| Dwarf ♀ X A136♂ | 107 | 88 | 1.22:1 | 1.851 | .20-.10 |
| Summary | 130 | 101 | 1.28:1 | 3.641 | .10-.05 |
| Test for homogeneity 1 degree of freedom | 4.628 - 3.641 = | | | .987 | .50-.30 |

1 normal:1 dwarf when backcrossed to a dwarf, + ¼ non-segregating dwarf plants (aaaa).

A small number of the normal plants from the backcross progeny of Dwarf X Clone 936-16 was selfed. A plant, A136, was found that segregated approximately 3 normal:1 dwarf. The genetic analyses of the self and

backcross ratios of this plant are given in Table 3 and Table 4.

A cross between a duplex (AAaa) and a simplex (Aaaa) plant would be expected to produce a progeny composed of 11 normal:1 dwarf if there is random chromosome segregation. Clone 936-16 was crossed with plant A136 and the results are shown in Table 5. The ratios fit the expected 11:1 ratio reasonably well. The lack of homogeneity between reciprocal crosses indicates that some selfing probably occurred, even though vacuum emasculation was practiced.

The critical test for tetrasomic inheritance was the analysis of the self progeny of plant A136. Since this plant segregated 3 normal: 1 dwarf, all of the normal plants should produce dwarfs upon backcrossing to the dwarf, if there is tetrasomic inheritance. Fifty of the normal segregates of the self progeny of plant A136 were testcrossed using the normal segregates as the female and dwarf plants as the male. (Crosses among dwarf segregates of Clone 936-16 and self progeny of these dwarf segregates had shown that dwarf plants are homozygous recessive. Only dwarf plants were obtained from dwarf X dwarf crosses.)

Table 5. Genetic analysis of progenies produced from reciprocal crosses of plants hypothesized to be duplex (AAaa) and simplex (Aaaa)

| | Progenies segregating approximately 11 normal:1 dwarf | | | | |
|---|---|---|---|---|---|
| | Normal | Dwarf | Observed ratio | $X^2$ | P |
| Clone 936-16 ♀ X A136♂ | 414 | 28 | 14.79:1 | 2.244 | .20-.10 |
| A136♀ X Clone 936-16♂ | 57 | 11 | 5.18:1 | 5.554 | .02-.01 |
| Summary | 471 | 39 | 12.08:1 | .286 | .70-.50 |
| Test for homogeneity: 1 degree of freedom | 7.798 - .286 | | | 7.512 | < .01 |

Table 6 is a summary of these test crosses. Of the 50 normal plants tested, 34 segregated approximately 1 normal:1 dwarf. The remaining 16 normal plants segregated at ratios that deviated somewhat from the expected 5 normal:1 dwarf ratio. These deviations will be discussed later. The fact that all 50 plants tested segregated for dwarfs upon test crossing is conclusive evidence of tetrasomic inheritance in alfalfa. The ratio of the plants segregating approximately 1:1 and approximately 5:1 is 34:16 which fits the expected 2:1 ratio predicted under tetrasomic inheritance.

Evidence for preferential pairing

There was an excess of normal plants in the test cross progenies of the duplex plants (AAaa). Double reduction or numerical non-disjunction would result in an excess of dwarfs, so these two meiotic variables were eliminated as a cause for the observed deviations from the expected 5:1 ratio.

Possible causes for the excess of normal segregates are as follows:

1. poor survival of dwarf plants
2. a certain percentage of selfing
3. differential transmission of gametes on the female side. Duplex plants can produce three kinds of gametes (AA + 4Aa + aa) whereas simplex plants normally produce only two kinds (Aa + aa).
4. preferential pairing of a homogenic nature.

Each possibility is discussed in the order listed.

Poor survival of the dwarf plants can be ruled out as a cause for excess of normal segregates because there was no other evidence of reduced viability of the dwarfs. By computing the effects of differential

Table 6. Genetic analysis of test cross ratios of normal segregates of the self progeny of plant A136 (in all cases dwarf plants used as male test cross parent)

| | Progenies segregating approximately 1 normal:1 dwarf | | | | |
|---|---|---|---|---|---|
| Normal segregate (female) | Normal | Dwarf | Observed ratio | $X^2$ | P |
| A136-87 | 6 | 12 | .50:1 | 2.000 | .20-.10 |
| -77 | 9 | 17 | .52:1 | 2.462 | .20-.10 |
| -71 | 9 | 16 | .56:1 | 1.960 | .20-.10 |
| -64 | 8 | 14 | .57:1 | 1.636 | .30-.20 |
| -67 | 33 | 49 | .67:1 | 3.121 | .10-.05 |
| -62 | 70 | 93 | .75:1 | 3.245 | .10-.05 |
| -54 | 14 | 18 | .78:1 | .500 | .50-.30 |
| -47 | 37 | 46 | .80:1 | .976 | .50-.30 |
| -73 | 40 | 47 | .85:1 | .563 | .50-.30 |
| -57 | 19 | 22 | .86:1 | .220 | .70-.50 |
| -70 | 41 | 46 | .89:1 | .287 | .70-.50 |
| -12 | 57 | 64 | .89:1 | .405 | .70-.50 |
| -78 | 10 | 11 | .91:1 | .048 | .90-.80 |
| -19 | 17 | 18 | .94:1 | .029 | .90-.80 |
| -50 | 52 | 54 | .96:1 | .038 | .90-.80 |
| -18 | 18 | 18 | 1.00:1 | .000 | 1.00 |
| -48 | 67 | 65 | 1.03:1 | .030 | .90-.80 |
| -61 | 76 | 74 | 1.03:1 | .027 | .90-.80 |
| -60 | 45 | 42 | 1.07:1 | .103 | .80-.70 |
| -41 | 30 | 28 | 1.07:1 | .069 | .80-.70 |
| -36 | 38 | 34 | 1.12:1 | .222 | .70-.50 |
| -75 | 18 | 16 | 1.13:1 | .118 | .80-.70 |
| -27 | 48 | 42 | 1.14:1 | .400 | .70-.50 |
| -84 | 15 | 13 | 1.15:1 | .143 | .80-.70 |
| -3 | 50 | 43 | 1.16:1 | .527 | .50-.30 |
| -26 | 50 | 42 | 1.19:1 | .696 | .50-.30 |
| -81 | 33 | 27 | 1.22:1 | .600 | .50-.30 |
| -13 | 11 | 9 | 1.22:1 | .200 | .70-.50 |
| -63 | 28 | 20 | 1.40:1 | 1.333 | .30-.20 |
| -10 | 33 | 23 | 1.43:1 | 1.785 | .20-.10 |
| -52 | 35 | 24 | 1.45:1 | 2.050 | .20-.10 |
| -83 | 12 | 7 | 1.71:1 | 1.316 | .30-.20 |
| -7 | 43 | 25 | 1.72:1 | 4.764 | .05-.02 |
| -68 | 33 | 19 | 1.74:1 | 3.769 | .10-.05 |
| Summary | 1105 | 1098 | 1.01:1 | .022 | .90-.80 |
| Test for homogeneity: 35.642 - .022 = 33 degrees of freedom | | | | 35.620 | .30-.20 |

Table 6. (Continued)

| Normal segregate (female) | Progenies segregating approximately 5 normal:1 dwarf | | | | |
|---|---|---|---|---|---|
| | Normal | Dwarf | Observed ratio | $X^2$ | P |
| A136-59 | 27 | 10 | 2.70:1 | 2.817 | .10-.05 |
| -24 | 45 | 12 | 3.75:1 | .776 | .50-.30 |
| -20 | 130 | 30 | 4.33:1 | .584 | .50-.30 |
| -28 | 32 | 7 | 4.57:1 | .044 | .90-.80 |
| -56 | 44 | 9 | 4.88:1 | .029 | .90-.80 |
| -79 | 16 | 3 | 5.33:1 | .011 | .95-.90 |
| -1 | 99 | 14 | 7.07:1 | 1.509 | .30-.20 |
| -11 | 81 | 11 | 7.36:1 | 1.486 | .30-.20 |
| -14 | 95 | 12 | 7.91:1 | 2.315 | .20-.10 |
| -25 | 144 | 17 | 8.47:1 | 4.366 | .05-.02 |
| -38 | 81 | 9 | 9:1 | 2.904 | .10-.05 |
| -21 | 76 | 7 | 10.82:1 | 4.076 | .05-.02 |
| -72 | 47 | 4 | 11.75:1 | 2.869 | .10-.05 |
| -45 | 49 | 3 | 16.33:1 | 4.461 | .05-.02 |
| -74 | 31 | 2 | 15.5:1 | 2.684 | .20-.10 |
| -23 | 175 | 10 | 17.5:1 | 16.870 | < .01 |
| Summary | 1172 | 160 | 7.33:1 | 21.041 | < .01 |
| Test for homogeneity: 15 degrees of freedom | | | 47.801 - 21.041 = | 26.760 | .05-.02 |

survival of dwarfs and normals it can be shown that survival of the dwarfs would have to be 70 percent of the normal to account for a 7.33:1 ratio in the test cross progeny of the duplex plants. Under this condition a ratio of 1.43:1 would be expected for the test cross progeny of the simplex plants. The observed 1.01:1 ratio deviates significantly from this hypothetical ratio and agrees almost perfectly with the expected considering equal viability of normal and dwarf plants.

Selfing can also be eliminated as the primary cause of the observed deviations although some selfing may have occurred. There was evidence of selfing in the reciprocal crosses between clone 936-16 and A136. The same argument can be used to eliminate selfing as a cause for the excess of normals as was used in the case of low survival. But in addition to this argument there is also direct evidence that selfing could not have been adequate to account for the observed deviations from a 5:1 ratio. Table 7 is a comparison of self fertility, cross fertility, and segregating ratios. From this table it can be observed that self fertility of the duplex plants is not higher than the self fertility of the simplex plants. Some of the plants with the most divergent ratios are practically self sterile. The ratio of self fertility to cross fertility is very low and by computation it can be shown that at least 30 percent selfing would be needed to account for a 7.33:1 ratio.

Differential transmission of the gametes can not be eliminated as a possible cause of the excess of normal segregates. As a rule, the duplex plant can produce one kind of gamete (AA) that the simplex plant can not. It is within the realm of possibility that this gamete has an

Table 7. Comparisons of self fertility, cross fertility with emasculation of the female, and ratio of Normal:Dwarf

| Plants segregating 1:1 | Self fertility | | | Cross fertility | | | Segregating ratio[a] |
|---|---|---|---|---|---|---|---|
| | Flowers | Seed | S/F[b] | Flowers | Seed | S/F[b] | |
| 136-77 | 34 | ø | .00 | 76 | 38 | .50 | .52:1 |
| -64 | 3 | ø | .00 | 52 | 35 | .67 | .57:1 |
| -67 | 40 | 6 | .15 | 81 | 89 | 1.10 | .67:1 |
| -73 | 52 | 10 | .19 | 47 | 120 | 2.55 | .85:1 |
| -70 | 100 | ø | .00 | 80 | 100 | 1.25 | .89:1 |
| -75 | 10 | ø | .00 | 47 | 43 | .91 | 1.13:1 |
| -81 | 88 | 30 | .34 | 62 | 76 | .12 | 1.22:1 |
| -83 | 48 | 7 | .15 | 41 | 28 | .68 | 1.71:1 |
| -68 | 13 | 1 | .08 | 46 | 73 | 1.58 | 1.74:1 |
| Summary | 388 | 54 | .14 | 532 | 602 | 1.13 | .93:1 |
| Plants segregating 5:1 | | | | | | | |
| 136-24 | 75 | ø | .00 | 59 | 59 | 1.00 | 3.75:1 |
| -79 | 36 | ø | .00 | 82 | 26 | .32 | 5.33:1 |
| -14 | 55 | 2 | .04 | 139 | 109 | .78 | 7.91:1 |
| -25 | 70 | 1 | .01 | 115 | 158 | 1.37 | 8.47:1 |
| -38 | 42 | ø | .00 | 141 | 93 | .66 | 9:1 |
| -21 | 29 | 1 | .03 | 79 | 83 | 1.05 | 10.82:1 |
| -72 | 82 | 9 | .11 | 46 | 62 | 1.35 | 11.75:1 |
| -45 | 3 | ø | .00 | 31 | 52 | 1.68 | 16.33:1 |
| -23 | 45 | ø | .00 | 174 | 187 | 1.07 | 17.5:1 |
| -74 | 18 | 3 | .17 | 37 | 25 | .67 | - |
| Summary | 455 | 16 | .04 | 903 | 854 | .95 | 9.68:1 |

[a]Normal:Dwarf

[b]Seed per flower

advantage and unites with the sperm more often than would be expected based upon its frequency. It should be pointed out, however, that the dwarf plants, capable of producing only homozygous recessive gametes, are fully fertile both as male and female parents.

Preferential pairing of the chromosomes during meiosis is a likely cause of the observed excess of normal plants. If this pairing is of a homogenic nature, an excess of heterogenic gametes (Aa) will be produced. These heterogenic gametes will combine with the homogenic gametes of the male parent to produce an excess of normal simplex plants (Aaaa) in the test cross progenies.

Although preferential pairing is considered the most likely cause of the excess of normal plants in the test cross ratios, differential transmission of the gametes cannot be ruled out. Only further progeny testing can provide the answer. If preferential pairing is the cause, an excess of simplex individuals will be found in the test cross progenies that show wide ratios of normal:dwarf. If differential transmission is the cause, an excess of duplex individuals will be found in the test cross progenies that show wide ratios of normal:dwarf.

## Part 2: The Inheritance of Dwarf Character in Source B

The seven $F_1$ plants of source B were selfed and the segregation ratios of the $F_2$ progenies are given in Table 8. Several different genetic models can be fitted equally well to these data. For example, a digenic-tetrasomic model, a quadrigenic-disomic model and a combination

Table 8. Segregating ratios in the $F_2$ progenies of $F_1$ plants from a cross, Dwarf X Normal, (these $F_1$'s are a source of dwarf plants designated source B)

| $F_1$ plants | $F_2$ progenies | | |
|---|---|---|---|
| | Normal | Dwarf | Ratio |
| $B_1$ | 538 | 2 | 269:1 |
| $B_2$ | 295 | 5 | 59:1 |
| $B_3$ | 1972 | 12 | 164:1 |
| $B_4$ | 607 | 1 | 607:1 |
| $B_5$ | 749 | 3 | 250:1 |
| $B_7$ | 182 | 2 | 91:1 |
| $B_9$ | 1260 | 23 | 55:1 |

of these two models can explain the data with a high probability. In addition, one can hypothesize a mono-genic model with varying degrees of homogenic preferential pairing to explain the various ratios. These models were not tested beyond the $F_2$.

Instead, plant B119, which segregated 3 normal:1 dwarf upon selfing, was selected from a small backcross progeny of Dwarf X B9. This plant was considered heterozygous for a single recessive gene controlling dwarf phenotype and provided an excellent basis for testing the hypothesis that alfalfa behaves genetically as an autotetraploid.

Plant B9 was hypothesized to be duplex (BBbb) for a recessive gene controlling dwarf phenotype. Plant B119 was hypothesized to be simplex

(Bbbb) for this same gene. Segregating ratios from these plants were tested and found to be in agreement with this hypothesis. The results are given in Table 9. There was, however, a deficiency of dwarf plants and an excess of normals. This deficiency was significant at the .05 level of probability in the selfed progeny of plant B9.

A critical test for tetrasomic inheritance is the genetic analysis of the selfed progeny of a plant believed to be simplex. For this test, 81 normal segregates were selected at random from the self progeny of plant B119. These plants were grown to maturity and selfed. Due to self incompatibility, only 43 of these plants produced enough seed for testing. Only plants that produced over 18 selfed seed were progeny tested.

If B119 is a simplex plant (Bbbb), and tetrasomic inheritance is the rule in alfalfa, 2/3 of the 43 plants tested should segregate 3 normal:1 dwarf. The remaining 1/3 should segregate 35 normal:1 dwarf. Table 10 presents the results of this test. Twenty-eight of the 43 plants, approximately 2/3 of the total, segregated approximately 3 normal:1 dwarf. This result would be expected from both disomic or tetrasomic inheritance. It is the remaining 15 plants that provide the critical evidence.

Eleven of the 15 plants produced self progenies that segregated approximately 35:1. Four plants did not segregate. In summary, 39 of the 43 plants tested segregated for dwarfs and the ratios obtained could occur only under tetrasomic inheritance.

The four plants that did not segregate probably were duplex plants (BBbb) that did not segregate due to small numbers in the segregating progenies. The average progeny size from the duplex plants is 97. Since the expected segregation of a duplex plant is 35:1, the probability of

Table 9. Genetic analysis of two plants heterozygous for dwarf genes; plant B9 assumed to be duplex (BBbb), plant B119 assumed to be simplex (Bbbb)

| | Ratios of selfed progenies | | | | | |
|---|---|---|---|---|---|---|
| | Normal | Dwarf | Observed ratio | Expected ratio | $X_2$ | P |
| B9 | 1260 | 23 | 55:1 | 35:1 | 4.781 | .05-.02 |
| B119 | 208 | 65 | 3.20:1 | 3:1 | .206 | .70-.50 |
| | Ratios of backcross progenies | | | | | |
| Dwarf ♀ X B9♂ | 919 | 158 | 5.82:1 | 5:1 | 3.190 | .10-.05 |
| Dwarf ♀ X B119♂ | 57 | 42 | 1.36:1 | 1:1 | 2.272 | .20-.10 |
| | Ratios of cross progenies | | | | | |
| B9 ♀ X B119♂ | 44 | 2 | 22:1 | 11:1 | .944 | .50-.30 |
| B119 ♀ X B9♂ | 95 | 9 | 10.56:1 | 11:1 | .017 | .90-.80 |
| Summary | 139 | 11 | 12.64:1 | 11:1 | .184 | .70-.50 |
| Test for homogeneity: 1 degree of freedom | .961 - .184 = | | | | .777 | .50-.30 |

Table 10. Genetic analysis of $F_3$ progenies from normal $F_2$ segregates selected at random from the self progeny of plant B119

| $F_2$ plant | Progenies segregating approximately 3 normal:1 dwarf | | | | |
|---|---|---|---|---|---|
| | $F_3$ progeny | | Observed ratio | $X^2$ | P |
| | Normal | Dwarf | | | |
| B119-33 | 95 | 48 | 1.98:1 | 5.597 | .02-.01 |
| -68 | 18 | 9 | 2.00:1 | 1.000 | .50-.30 |
| -24 | 34 | 16 | 2.12:1 | 1.307 | .30-.20 |
| -29 | 25 | 11 | 2.27:1 | .593 | .50-.30 |
| -31 | 176 | 74 | 2.38:1 | 2.821 | .10-.05 |
| -28 | 20 | 8 | 2.50:1 | .190 | .70-.50 |
| -37 | 38 | 15 | 2.53:1 | .308 | .70-.50 |
| -61 | 18 | 7 | 2.57:1 | .120 | .80-.70 |
| -65 | 29 | 11 | 2.64:1 | .133 | .80-.70 |
| -77 | 36 | 12 | 3.00:1 | .000 | 1.00 |
| -40 | 46 | 14 | 3.29:1 | .089 | .80-.70 |
| -71 | 32 | 9 | 3.56:1 | .203 | .70-.50 |
| -39 | 29 | 8 | 3.63:1 | .225 | .70-.50 |
| -52 | 62 | 17 | 3.65:1 | .513 | .50-.30 |
| -22 | 37 | 10 | 3.70:1 | .348 | .70-.50 |
| -30 | 112 | 30 | 3.73:1 | 1.136 | .30-.20 |
| -63 | 30 | 8 | 3.75:1 | .316 | .70-.50 |
| -25 | 132 | 35 | 3.77:1 | 1.455 | .30-.20 |
| -26 | 19 | 5 | 3.80:1 | .222 | .70-.50 |
| -3 | 311 | 80 | 3.89:1 | 4.297 | .05-.02 |
| -20 | 121 | 31 | 3.90:1 | 1.719 | .20-.10 |
| -43 | 39 | 10 | 3.90:1 | .552 | .50-.30 |
| -50 | 47 | 12 | 3.92:1 | .684 | .50-.30 |
| -10 | 47 | 12 | 3.92:1 | .684 | .50-.30 |
| -62 | 56 | 14 | 4.00:1 | .933 | .50-.30 |
| -38 | 116 | 26 | 4.46:1 | 3.390 | .10-.05 |
| -17 | 60 | 10 | 6.00:1 | 4.286 | .05-.02 |
| -16 | 47 | 7 | 6.57:1 | 4.173 | .05-.02 |
| Summary | 1832 | 549 | 3.34:1 | 4.173 | .05-.02 |
| Test for homogeneity: 37.294 - 4.791 = 27 degrees of freedom | | | | 32.503 | .30-.20 |

Table 10. (Continued)

| $F_2$ plant | Progenies segregating approximately 35 normal:1 dwarf | | | | |
|---|---|---|---|---|---|
| | $F_3$ progeny | | Observed | $X^2$ | P |
| | Normal | Dwarf | | | |
| B119-36 | 72 | 4 | 18:1 | 1.690 | .20-.10 |
| -41 | 322 | 17 | 18.94:1 | 6.114 | .02-.01 |
| -23 | 147 | 7 | 21:1 | 1.720 | .20-.10 |
| -27 | 90 | 4 | 22.50:1 | .736 | .50-.30 |
| -59 | 119 | 3 | 39.67:1 | .054 | .90-.80 |
| -32 | 83 | 2 | 41.50:1 | .061 | .90-.80 |
| -78 | 43 | 1 | 43:1 | .042 | .90-.80 |
| -14 | 59 | 1 | 59:1 | .319 | .70-.60 |
| -15 | 120 | 2 | 60:1 | .608 | .50-.30 |
| -58 | 62 | 1 | 62:1 | .339 | .70-.50 |
| -21 | 86 | 1 | 86:1 | .872 | .50-.30 |
| -2 | 92 | 0 | - | | |
| -12 | 71 | 0 | - | | |
| -7 | 57 | 0 | - | | |
| -35 | 30 | 0 | - | | |
| Summary | 1453 | 43 | 33.79:1 | .030 | .90-.80 |

having a non-segregating progeny is given by the following expression:

$$P = C_r^n \, p^{n-r} q^r$$

$$P = \frac{35}{36}^{(97)} = .053.$$

If the probability of a progeny not segregating is .053, then the probability that 4 or more progenies out of 15 will not segregate is

$1 - \{P[15 \text{ out of } 15 \text{ will segregate}] + P[14 \text{ out of } 15 \text{ will segregate}] + P[13 \text{ out of } 15 \text{ will segregate}] + P[12 \text{ out of } 15 \text{ will segregate}]\} = .008.$

The true probability is actually somewhat higher than this value because of the unequal progeny sizes. If one uses the average of the non-segregating progenies (n = 63) for the computations, the probability of 4 or more progenies out of 15 not segregating is .177. The true probability lies between these two limits ($.008 < P < .177$).

There was an excess of normals in the progenies of the 28 simplex (Bbbb) plants. The ratio, 3 normal:1 dwarf, can be rejected at the .05 level of probability. Preferential pairing does not affect the segregating ratios of simplex plants and can be eliminated as a possible explanation for the excess of normal segregates. Poor survival of the homozygous recessive or inadequate transmission of the homogenic gametes could account for this deficiency of dwarf plants.

# DISCUSSION

The symbols $Dw_1$ and $dw_1$ are suggested for the dominant and recessive alleles of the gene controlling dwarf phenotype in source A, according to the procedure outlined by Myers (1948) for designating genes in alfalfa. The symbols $Dw_2$ and $dw_2$ are suggested for the dominant and recessive alleles of the gene analyzed in source B.

Stanford and Clement (1958) stated that tetrasomic ratios should be the rule in alfalfa, but the probability of disomic ratios associated with certain chromosomes is not excluded. Later, Cleveland and Stanford (1959) concluded that whatever the origin of alfalfa, the species as it exists today, deviates in chromosome behavior from that of a true autoploid. Tysdal et al. (1942) had already suggested that alfalfa may be intermediate between a true alloploid and true antoploid.

In the analysis of the inheritance of dwarf character in source A, strong evidence was found indicating preferential pairing of a homogenic nature. However, preferential pairing was not proven. Assuming that preferential pairing did occur, this would support the theory that alfalfa has a tendency to deviate from true autotetraploid behavior in the direction of allotetraploid behavior.

In the past, the terms disomic inheritance and tetrasomic inheritance have had very specific meanings. Disomic has been used to indicate complete preferential pairing in allopolyploids while tetrasomic has suggested random pairing among homologs in autotetraploids. Little (1945) and Buzzell (1965) have shown how deviations from these well defined situations may occur. The question now arises: How valid are these terms if devia-

tions in pairing behavior are known? The average ratio of $H_o$:$H_e$ in the test cross progenies of source A, assuming that preferential pairing was the cause of the excess of normals, was .5:.5. With strict disomic inheritance the ratio of $H_o$:$H_e$ is either 1:0 or 0:1 and with strict tetrasomic inheritance the ratio of Ho:He is 1/3:2/3.

In the absence of proof of preferential pairing, the type of inheritance found for $Dw_1$ is referred to as tetrasomic.

The data from the study of $Dw_2$ indicated tetrasomic inheritance in alfalfa, but the data were not extensive enough to detect slight deviations in chromosome pairing that may have occurred.

## SUMMARY

Two sources of plant material, designated A and B, from Medicago sativa L. were studied. Segregating progenies from both of these sources contained plants with dwarf phenotypes.

In source A the dwarf phenotype was conditioned by a single recessive gene inherited tetrasomically. Dwarf plants were nulliplex and normal plants were either simplex, duplex, triplex or quadruplex.

In source B the dwarf phenotype was conditioned by one or more recessive genes. The segregation of a single gene was observed in the $F_2$ and $F_3$ generations of a simplex plant selected from a backcross progeny. Tetrasomic inheritance was found.

Crosses between dwarfs from source A and dwarfs from source B produced only normal offspring indicating that independent genetic systems were operating in the two sources of plant material.

The symbols $Dw_1$ and $dw_1$ were suggested for dominant and recessive alleles of the gene controlling dwarf phenotype in source A and the symbols $Dw_2$ and $dw_2$ were suggested for the dominant and recessive alleles of the gene analyzed in source B.

Deviations from the expected segregating ratios, based upon random chromosome segregation of an autotetraploid, were found. Preferential pairing of chromosomes was suggested as the cause of an excess of normal plants in progenies of duplex plants from source A. Also, differential transmission of the different gametes, $Dw_1Dw_1$, $Dw_1dw_1$, and $dw_1dw_1$, with the homozygous dominant gamete having an advantage, was suggested as an alternative possibility.

## LITERATURE CITED

Armstrong, J. M. 1954. Cytological studies in alfalfa polyploids. Canadian Journal of Botany 32: 531-542.

Armstrong, J. M. and Gibson, D. R. 1941. Inheritance of certain characters in the hybrid of Medicago media and Medicago glutinosa. Scientific Agriculture 22: 1-10.

Atwood, S. S. and Grunn, P. 1951. Cytogentics of alfalfa. Bibliographia Genetica 14: 133-188.

Barnes, D. K. and Hovin, A. W. 1965. Inheritance of a pale-green plant color mutant in alfalfa. Crop Science 5: 187-188.

Bartlett, M. S. and Haldane, J.B.S. 1934. The theory of inbreeding in autotetraploids. Journal of Genetics 29: 175-180.

Bauder, W. W. 1938. The inheritance of the odd-leaf character in Medicago sp. Unpublished M.S. thesis. Library, University of Nebraska, Lincoln, Nebraska. Original not available for examination; cited in Atwood, S. S. and Grunn, P. 1951. Cytogenetics of alfalfa. Bibliographic Genetica 14:163.

Burkart, A. 1937. Frecuencia de la fecundacion cruzada en la alfalfa, segun experiencias con plantas recesivas de flor blanca y consideraciones sobre el mejoramiento de esta forrajera. Revista Argentina de Agronomia 4: 171-196. Original not available for examination; cited in Atwood, S. S. and Grunn, P. 1951. Cytogenetics of alfalfa. Bibliographic Genetica 14: 159.

Burnham, C. R. 1962. Discussions in cytogenetics. Burgess Publishing Company, Minneapolis 15, Minnesota.

Burton, G. W. 1937a. The inheritance of various morphological characters in alfalfa and their relation to plant yields in New Jersey. New Jersey Agricultural Experiment Station Bulletin 628.

Burton, G. W. 1937b. Alfalfa inheritance studies in New Jersey. Journal of American Society of Agronomy 29: 600-606.

Busbice, T. H. and Wilsie, C.P. 1966. Inbreeding depression and heterosis in autotetraploids with application to Medicago sativa L. [To be published in Euphytica. ca. 1966].

Buzzell, R. I. 1965. Effects of variation in bivalent preferential pairing. [To be published in Canadian Journal of Genetics and Cytology. ca. 1965].

Buzzell, R. I. and Wilsie, C. P. 1963. Genetic investigations of brown keel tip color in Lotus corniculatus L. Crop Science 3: 128-130.

Carnahan, H. L. 1960. Some theoretical considerations of the consequences of multiple alleles in relation to inbreeding and testing procedures in autopolyploids. Alfalfa Improvement Conference Report 17: 19-22.

Catcheside, D. G. 1956. Double reduction and numerical non-disjunction in tetraploid maize. Heredity 10: 205-218.

Childers, W. R. 1962. The nature and inheritance of a yellow-leaf character in Medicago sativa L. Canadian Journal of Botany 40: 89-93.

Childers, W. R. and McLennan, H. A. 1960. Inheritance studies of a complete male sterile character in Medicago sativa L. Canadian Journal of Genetics and Cytology 2: 57-65.

Childers, W. R. and McLennan, H. A. 1961. The inheritance and histology of a chlorophyll-deficient character in Medicago sativa L. Canadian Journal of Botany 39: 847-853.

Clement, W. M. and Lehman, W. F. 1962. Fertility and cytological studies of a dihaploid plant of alfalfa, Medicago sativa L. Crop Science 2: 451-453.

Clement, W. M. and Stanford, E. H. 1961. Chromatic and achromatic chromosome segments and their relation to chiasma frequency in alfalfa, Medicago sativa L. Genetics 46: 859.

Cleveland, R. W. and Stanford, E. H. 1959. Chromosome pairing in hybrids between Medicago sativa L. and diploid Medicago falcata L. Agronomy Journal 51: 488-492.

Comstock, R. E. 1952. Statistical genetics in alfalfa breeding. Alfalfa Improvement Conference Report 13: 28-30.

Covas, G. and Fernandez, O. 1947. Cruzamientos naturales y artificiales en alfalfa. Revista Argentina de Agronomia 14: 70-72. Original not available for examination; cited in Atwood, S. S. and Grunn, P. 1951. Cytogenetics of alfalfa. Bibliographic Genetica 14: 161.

Davis, R. L. 1956. Tetrasomic inheritance of an elongated hypocotyl mutant in alfalfa. Agronomy Journal 48: 197-199.

Demarly, Y. 1963. Genetique des tetraploides et amelioration des plantes. Theses presentees a la faculte des sciences de L'Universite de Paris. Institut National de la Recherche Agronomique, 149, rue de Grenelle, Paris.

Dessureaux, L. 1961. Genes controlling yellow cotyledons in alfalfa. Forage Notes (Canada) 7(3): 27.

Doyle, G. G. 1963. Preferential pairing in structural heterozygotes of Zea mays. Genetics 48: 1011-1027.

Dudley, J. W. 1964. A genetic evaluation of methods of utilizing heterozygosis and dominance in autotetraploids. Crop Science 4: 410-413.

Dudley, J. W. and Wilsie, C. P. 1956. Inheritance of branched inflorescence and vestigal flower in alfalfa, Medicago sativa L. Agronomy Journal 48: 47-50.

Fyfe, J. L. and Wills, A. B. 1955. Disturbed segregations of an albino in lucerne. Heredity 9: 291.

Gilles, A. and Randolph, L. F. 1951. Reduction of quadrivalent frequency in autotetraploid maize. American Journal of Botany 38: 12-17.

Goplen, B. P. and Stanford, E. H. 1960. Autotetraploidy and linkage in alfalfa: a study of resistance to two species of root-knot nematodes. Agronomy Journal 52: 337-342.

Grun, P. 1951. Variations in the meiosis of alfalfa. American Journal of Botany 38: 475-482.

Hagem, O. 1919. Einige $F_2$ and $F_3$ Generationen bei dem Bastard Medicago sativa X M. falcata. Nyt Magazin Naturvidenskaberne 56: 149-165.

Haldane, J.B.S. 1930. Theoretical genetics of autopolyploids. Journal of Genetics 22: 359-372.

Hanson, A. A. 1952. The nature of polyploidy in alfalfa. Alfalfa Improvement Conference Report 13: 20-21.

Julen, G. 1944. Investigations on diploid, triploid and tetraploid lucerne. Hereditas 30: 567-582.

Kempthorne, O. 1955. The correlation between relatives in random mating populations. Cold Spring Harbor Symposium on Quantitative Biology 20: 60-75.

Korohoda, J. 1933. (Inheritance of certain characters of the hybrid of Medicago sativa and Medicago falcata) Panstwowy Instytut Naukowego Gospodarstwa Wiejskiego W Pulawach, Krakow Pamplet 14: 378-393.

Ledingham, G. F. 1940. Cytological and developmental studies of hybrids between Medicato sativa and a diploid form of M. falcata. Genetics 25: 1-15.

Lepper, R., Jr. and Odland, T. E. 1939. Inheritance of flower color in alfalfa. Journal American Society of Agronomy 31: 209-216.

Lesins, K. 1952. Some data on the cytogenetics of alfalfa. Journal of Heredity 43: 287-291.

Lesins, K. 1957. Cytogenetic study of a tetraploid plant at the diploid level. Canadian Journal of Botany 35: 181-196.

Levings, C. S. and Dudley, J. W. 1963. Evaluation of certain mating designs for estimation of genetic variance in autotetraploid alfalfa. Crop Science 3: 532-535.

Lewis, W. M. and Elling, L. J. 1956. The inheritance of a lethal character in alfalfa. Alfalfa Improvement Conference Report 15: 7-9.

Li, C. C. 1957. Genetic variance of autotetraploids with 2 alleles. Genetics 42: 583-592.

Little, T. M. 1945. Gene segregations in autotetraploids. Botanical Review 11: 60-85.

Little, T. M. 1958. Gene segregations in autotetraploids. II. Botanical Review 24: 318-339.

MacVicar, R. M. 1935. Inheritance of seed color in alfalfa. Scientific Agriculture 15: 314-328.

Markus, R. and Wilsie, C. P. 1957. Inheritance of an exposed stigma mutant in alfalfa, Medicago sativa L. Agronomy Journal 49: 604-609.

Mather, K. 1935. Reductional and equational separations of the chromosomes in bivalents and multivalents. Journal of Genetics 30: 53-57.

Mather, K. 1936. Segregation and linkage in autotetraploids. Journal of Genetics 32: 287-314.

Mather, K. 1957. The measurement of linkage in heredity. John Wiley and Sons, Inc. New York.

Menzel, M. Y. 1964. Preferential chromosome pairing in allotetraploid Lycopersicon esculentum - Solanum lycopersicoides. Genetics 50: 855-862.

Murray, B. E. and Craig, I. L. 1962. A cytogenetic study of the X-ray induced cauliflower-head and single-leaf mutation in Medicago sativa L. Canadian Journal of Genetics and Cytology 4: 379-385.

Myers, W. M. 1948. Naming genetic characters of alfalfa. Alfalfa Improvement Conference Report 11: 43-44.

Odland, T. E. and Lepper, R., Jr. 1939. A crinkled leaf mutation in alfalfa. Journal of American Society of Agronomy 31: 128-130.

Oldemeyer, R. K. 1956. Inheritance of the white seed character in alfalfa. Agronomy Journal 48: 449-451.

Oldemeyer, R. K. and Brink, R. A. 1953. Effect on fertility at the tetraploid level of the genome derived from diploid Medicago falcata. Agronomy Journal 45: 598-601.

Pedersen, M. W. and Barnes, D. K. 1965. Inheritance of downy mildew resistance in alfalfa. Crop Science 5: 4-5.

Peltier, G. L. and Tysdal, H. M. 1934. Wilt and cold resistance of self-fertilized lines of alfalfa. Nebraska Agricultural Experiment Station Research Bulletin 76.

Schröck, O. 1943. Genetische Beobachtungen an Luzerne (Medicago media). Zeitschrift für Pflanzenzuchtung 25: 81-91.

Shaver, D. L. 1963. The effect of structural heterozygosity on the degree of preferential pairing in allotetraploids of Zea. Genetics 48: 515-524.

Soudah, R. E. 1962. Tetrasomic inheritance of flower color in alfalfa, Medicago sativa L. Dissertation Abstract 23: 1845.

Stanford, E. H. 1951. Tetrasomic inheritance in alfalfa. Agronomy Journal 43: 222-225.

Stanford, E. H. 1959. The zebra leaf character in alfalfa and its dosage-dominance relationship. Agronomy Journal 51: 274-277.

Stanford, E. H. 1965. Inheritance of sticky leaf character in alfalfa. Crop Science 5: 281.

Stanford, E. H. and Clement, W. M. 1958. Cytology and crossing behavior of haploid alfalfa plant. Agronomy Journal 50: 589-592.

Stanford, E. H. and Cleveland, R. W. 1954. The inheritance of two leaf abnormalities in alfalfa. Agronomy Journal 46: 203-206.

Stargaard, A. K. 1957. Genetics studies in alfalfa, Medicago sativa L. Dissertation Abstract 17: 203-204.

Twamley, B. E. 1955. Flower color inheritance in diploid and tetraploid alfalfa. Canadian Journal of Agricultural Science 35: 461-476.

Tysdal, H. M., Kiesselback, T. A. and Westover, H. L. 1942. Alfalfa breeding. Nebraska Agricultural Experiment Station Research Bulletin 124.

Waldron, L. R. 1925. Alfalfa bud mutation. Journal of Heredity 16: 423-424.

Waldron, L. R. 1929. Vererbung der Blütenfarbe bei Hybriden von Medicago sativa and Medicago falcata. Pflanzenbau 6: 157-166.

Weihing, R. M. 1948. Flower color inheritance in alfalfa. Journal of American Society of Agronomy 40: 746-50.

Whittington, W. J. and Burrage, W. S. 1963. Inheritance of a ruptured epidermis in alfalfa. Crop Science 3: 256-58.

Wilson, M. C. 1947. Inheritance of resistance in alfalfa to bacterial wilt. Journal of American Society of Agronomy 39: 570-583.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. C. P. Wilsie for his guidance during the course of the study. Appreciation is also extended to the members of the graduate committee, Dr. C. C. Bowen, Dr. K. J. Frey, Dr. W. E. Loomis, and Dr. D. S. Robertson, for their critical reading of the manuscript and helpful suggestions.